

Investigation of Food Additive Potential of the *Rosa damascena* Mill. (Isparta Rose): Vinegar with Probiotic Addition

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Research Article

Keywords: Vinegar, probiotic strains, *Rosa damascena* Mill, functional products

Posted Date: July 27th, 2023

DOI: <https://doi.org/10.21203/rs.3.rs-3194799/v1>

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Additional Declarations: No competing interests reported.

Version of Record: A version of this preprint was published at Revista Mexicana de Ingeniería Química on November 22nd, 2023. See the published version at <https://doi.org/10.24275/rmiq/Alim24156>.

Abstract

Rosa damascena Mill., named also 'Isparta rose', is grown in the province of Isparta in Turkey. It is an aromatic plant having sharp and intensive scent and rich in bioactive compounds. In the literature, it has been reported that Isparta rose has a potential as natural food additives. However, the application to be natural food additive has not been study detailed. Therefore, rose vinegar was produced by the traditional method in present study and analyzed reported. Moreover, by adding probiotic strains to these vinegars produced, quality characteristics of the vinegars such as physicochemical, microbiological and some bioactive compounds were determined during storage time. The presence of main aroma compound associated with a rose honey-like odor and volatile aroma compounds such as dodecene, tetradecanol, linalool as well as phenyl ethyl alcohol are determined in all the vinegars. The aroma components have increased the pleasant taste and acceptability of vinegars in terms of sensory properties. On the other hand, it is determined that various organic acids (93.43 mg/100 mL oxalic acid, 53.57 mg/100 mL propionic acid, 10.11 mg/100 mL fumaric acid) and aroma components produced by *Lactiplantibacillus plantarum* strain are significantly affected the lactic and acetic acid bacteria growth. Additionally, the fact that the strain has completed the storage period with 83.96% viability, it shows that the has including the required number of viable microorganisms for produced vinegar to be called a probiotic during this time. All the results showed that it has potential that vinegar with culture additives will also create an alternative to functional probiotic drinks.

Introduction

The popularity of traditional unpasteurized vinegar produced at home using various substrates with fermentable sugars has recently increased due to its health benefits. Research has indicated that the vinegar production methods have a significant impact on the bioactive components, suggesting that vinegar produced using traditional methods may exhibit higher functional properties compared to those produced industrially [1, 2] In Turkey, a wide range of vinegar is produced using different raw materials through both traditional and industrial processes [2].

The antioxidant and antimicrobial potential of vinegar is influenced by phenolic substances, which vary depending on the raw material and production process employed [2]. The *rosa* genus offers a variety of raw materials suitable for vinegar production, including approximately 200 species, among which *Rosa damascena*, a crucial species within the Rosacea family, holds particular significance [3]. *Rosa damascena* Mill., a perennial shrub native to Europe and the Middle East and known as 'Isparta Güllü' in Turkey, is recognized for its pink flowers[4, 5]. The petals and fruits of the rose plant contain abundant bioactive substances such as essential oils, tannins, carotenoids, anthocyanins, organic acids, vitamins, and minerals. The leaves of the rose plant, in particular, possess high levels of ellagic acid, making them a valuable natural source of phenolic antioxidants [6, 7]. The rose plant is also rich in phenolics, flavonoids, carotenoids, and anthocyanins [3, 8]. Traditionally, rose has been utilized to address various health issues, including constipation, depression, gastrointestinal disorders, and respiratory problems [9]. As the demand for functional foods continues to rise, rose has gained considerable attention from scientists, food manufacturers, and consumers as a novel source of functional food [10].

In vinegar production, the raw material undergoes a typical fermentation process where yeasts convert simple sugars into alcohol, followed by oxidation of the alcohol to acetic acid by acetic acid bacteria (AAB) [11]. Lactic acid bacteria (LAB), which are predominant in most fermented products [12], play a crucial role in enhancing the taste and aroma of vinegar while reducing its pH value [2, 13]. Moreover, LAB aids in preventing the growth of undesirable microorganisms [14, 15]. The organic acids produced by LAB disrupt the outer membrane of bacteria, hinder macromolecular synthesis, increase intracellular osmotic pressure, and promote the formation of antibacterial peptides [16].

Apart from the bioactive compounds, beneficial microorganisms in functional foods contribute to their nutritional value. The impact of microorganisms involved in fermentation on the development of functional products can even vary among strains of the same species. Therefore, rose vinegar is produced using a traditional method involving four different LAB strains (*Lactiplantibacillus plantarum*, *Limosilactobacillus fermentum*, *Weisella cibaria*, *Loigolactobacillus coryniformis*) with various probiotic properties. The vinegar samples are evaluated for their physicochemical properties (Brix, pH, total acidity,

color), microbiological composition (lactic acid bacteria, acetic acid bacteria, yeast-mold), total phenolic compounds, antioxidant activity, organic acid, and aroma composition, as well as sensory characteristics.

Material and metods

Materials

Samples of roses (*Rosa damascena* Mill.) from the provinces of Isparta, Turkey, were collected as raw material for vinegar production. Four different lactic acid bacteria (LAB) used in this study were isolated from various fermented cheeses, pickle and fresh fruits, as indicated in Table 1. These LAB strains have been previously characterized for their phenotypic descriptions such as Gram-staining, catalase–oxidase and mobility test, biochemical, and probiotic properties by [17], following the protocols outlined by [18]. Strains by sequence 16S rRNA gene sequencing, blast numbers and codes, are given in previous study by [19].

Table 1
The names of the vinegar groups and the lactic acid bacteria and their isolation source

Group name	Lactic acid bacteria content	Source
Control	Any strain wasn't added	
Group 1	<i>Lactiplantibacillus plantarum</i>	Pickle
Group 2	<i>Limosilactobacillus fermentum</i>	White cheese
Group 3	<i>Weisella cibaria</i>	Tulum cheese
Group 4	<i>Loigolactobacillus coryniformis</i>	Bitter orange

Production of probiotic-added traditional rose vinegar

The rose samples were first washed and crushed. Subsequently, three samples, each weighing 1000g, were placed in 5 L glass jars. Next, 1.5 L of water was added to each batch and stored at a temperature of + 4°C for one week. After this process, 0.3 g/L of yeast *Saccharomyces cerevisiae* (Kurumaya, Pakmaya, Turkey) and 50 g of honey (Özkovan, Turkey) were introduced into each jar. In order to complete the ethyl alcohol fermentation, the samples were sealed with their lids closed and kept for 20 days. After the alcohol fermentation, 150 mL of homemade vinegar was added to each jar, and the remaining volume was adjusted to 5 L by adding water. The lids of the jars were secured with a cloth to allow oxygen to enter. The vinegars were stored in a dark environment at a temperature range of 28–30°C and underwent acetic acid fermentation. Periodic measurements of acidity were performed throughout this period. The acetic acid fermentation process concluded after 60 days [20] and immediately after the samples were filtered, LAB bacteria were added to the relevant groups. It was left to ferment for 7 days. Table 1 provides the names of the rose vinegar groups produced using four different lactic acid bacteria (LABs), a control group in the Food Technology Laboratory of Burdur Mehmet Akif Ersoy University, and the corresponding LAB strains used. Figure 1 illustrates a schematic diagram depicting the process of adding starter cultures to rose vinegar. All vinegar production and analyses were made in triplicate and the mean values and standard deviations were calculated.

Determination of viability in rose vinegar of LAB and other microorganisms

Under aseptic conditions, 1 mL samples of rose vinegar were obtained from each group, and decimal dilutions were prepared. AAB counts were performed using Glucose-Yeast Extract-Calcium Carbonate Agar (GYC agar) obtained from Merck, Germany. The plates were then incubated at 30°C for 5–10 days, as described by [21]. LAB counts were determined using De

Man Rogosa and Sharpe (MRS) Agar from Merck, Germany, and incubated at 30°C for two days, following the method outlined by [22]. Potato Dextrose Agar (obtained from Merck, Germany) acidified with 10% tartaric acid (also from Merck, Germany) was employed to assess the mold-yeast counts in the vinegar samples. The plates were incubated at 25°C for 3–5 days, following the procedure described in FDA-BAM.

Determination of the physicochemical parameters of the rose vinegar

The °Brix values of the rose vinegar samples were determined using a digital refractometer (HANNA HI 96801, Germany). pH measurements of the vinegar samples were carried out using a pH meter (Mettler Toledo SG23-FK2, Switzerland). The vinegar samples' color values (L^* , a^* , b^*) were measured using a Color Spectrophotometer (3NH YS3020, China) based on the CIE-LAB system. The total acidity results of the samples were obtained by titrating with 0.1N NaOH and expressed as a percentage of acetic acid [23].

Total phenolic content

The total phenolic contents in rose vinegar were determined using the Folin-Ciocalteu colorimetric method, and the results were expressed in milligrams of gallic acid equivalent per liter (mg GAE/L) [24].

Determination of antioxidant activity

The total antioxidant activity of the samples was determined using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) analysis method, as described by Molyneux. Rose vinegar samples were diluted with 80% methanol and treated with the DPPH solution. The mixture was then allowed to stand for thirty minutes at room temperature in the dark. The absorbance values of the samples were measured using a spectrophotometer (Optizen Pop Nano Bio, Mecasys Co., Ltd., Korea) at a wavelength of 515 nm. The results were expressed as μmol Trolox equivalent per mL ($\mu\text{mol TE/mL}$) [25].

Determination of organic acids

The organic acid profile of vinegar samples was determined using the Thermo Scientific Ultimate 3000 UPLC and Thermo Scientific TSQ Fortis system (Thermo Fisher Scientific Inc., Waltham, Massachusetts, USA) at Suleyman Demirel University Innovative Technologies Center Application and Research Center (YETEM), following the method employed by [26]. In organic acid sample preparation, a Supelco C18 solid phase cartridge (Waters Associates, Ireland) was initially conditioned in 3 mL of methanol and washed with 10 mL of pure water. A 5-mL rose vinegar was mixed with 5 mL of 2% H_3PO_4 and filtered with coarse filter paper. From the resulting filtrate, 1 mL was diluted with 3 mL extraction solution (0.01 M KH_2PO_4 , pH 8.0). One milliliter of this diluted solution was passed through the cartridge, collecting the eluate in a tube. The cartridge was then washed with 2 mL of the extraction solution. The eluates were combined and a volume of 10 μL was injected into the HPLC. The analysis involved a chromatographic separation of organic acids on a Hypersil Gold RP C18 (1.9 μm), 50 x 2.1 mm UHPLC column (Thermo Fisher Scientific Inc., Waltham, Massachusetts, USA). Chromatographic evaluations were conducted using Xcalibur software. For the analyses, mobile phases consisting of 95% water and 5% methanol with 0.1% formic acid and 4 mM ammonium formate, as well as 95% methanol and 5% water with 0.1% formic acid and 4 mM ammonium formate, were employed. An isocratic flow rate of 0.6 mL/min was maintained, and each injection lasted for 25 minutes, with an injection volume of 10 μL . The HPLC column temperature was set at 40°C.

Determination of aroma volatiles

Volatile components in vinegar were determined by using gas chromatography mass spectrometry (Shimadzu GC-MS-2010 Plus, Japan) with solid phase micro-extraction technique (SPME). All the vinegars were filtered by 0.45 μm teflon filter. For this purpose, LAB added vinegar samples were placed in an amber headspace vial (Supelco 27159 15 mL clear PTFE /Silicone septa Cap) on the 7th day of incubation and closed with an airtight silicone/PTFE cap. After these vials were kept at 60°C for 30 minutes, volatile components were absorbed in the headspace with 75 μm thick Carboxene-Polydimethylsiloxane (CAR/PDMS) coated fused silica SPME fiber (Supelco, Bellefonte, PA, USA). Capillary column of Restek (Rx-5 Sil MS 30 m x 0.25 mm, 0.25 μm , catalog no: Restek 13623) was used in the device. GC-MS parameters of the method

used in our study; injection temperature: 250°C, column flow rate: 1.61 mL/min, column temperature reached 250°C in 4°C increments per minute after standing at 40°C for 2 minutes, and at 250°C 5 minutes was determined as waiting and used in the analysis. To identify the peaks obtained after injection, after entering the method parameters, the C7-C30 alkane series was injected into the device, respectively, and defined in four different (Wiley, Nist, Tutor and FFNSC) GC-MS libraries. The "Retention Index" (RI) values calculated using the retention times of each peak and the retention times of the hydrocarbon standard were taken as reference.

Sensory analysis

Vinegar samples were evaluated on the seventh day of fermentation by 12 female and 10 male panelists (Food Engineer, dietitian, doctor, student and cook) aged between 18–40 from the Gastronomy and Culinary Arts and Cookery Department of Ardahan and Ağrı İbrahim Çeçen University in Turkey. Experiment adhered to TSE (Turkish Standardization Institute) standards number 1880 EN 13188 for hedonic testing of vinegar in controlled environments (TSE, 2016). Panelists with illnesses like the flu, a cold, or allergic rhinitis who could make it difficult to assess the sample sensory were not allowed to participate. About 100 mL of the rose vinegar was dosed in glass and placed at room temperature until serving under white light. The evaluations were made in the mid-morning between 10:00 and 13:00 am. The overall acceptability of the vinegar samples was evaluated by considering odor and taste profiles with drinking (aromatic intensity, ethyl acetate odor, sharpness, wine character, yeast aroma and taste, bitterness, fluidity. The evaluation process employed a 9-point scale (ranging from 1 for very low to 9 for very high) for each evaluator to express the intensity of the specific characteristics (Gomez, 2006). The means and standard deviations for all attributes were determined for each sample shown to the participant in each session.

Statistical analysis

The experiments were conducted in triplicate, and the acquired data were reported as mean $\pm$ standard deviation. The statistical software Minitab 17 (Minitab, Inc., State College, PA, USA) was employed for data analysis. The differences were assessed using a one-way analysis of variance (ANOVA). The Tukey test was also employed to examine the effects of microorganisms utilized in vinegar production on specific vinegar properties.

Results and discussion

Viability of LAB and other microorganisms in rose vinegar during the fermentation

According to Fig. 2, the initial number of viable cells in all probiotic groups was 7.17 log CFU/mL. However, after three days of fermentation, there was a decrease of approximately 1 logarithm in all groups except Group 4. The average viable cell count for all groups at the end of this period was 6 logarithms. Unfortunately, Group 4 did not meet the required live probiotic microorganisms count by the third day's end. In the other groups, the count remained at 6 logarithms or higher. Group 4 had a survivability of 71.96% at the end of the period, while the other three groups showed higher survivability, averaging 84%.

The *Lpb. plantarum* DA100 strain exhibited a slight decrease in viability from 84.79–83.96% between the third and seventh days. The initial viability count, which was 7.17 log CFU/mL, decreased by approximately 1 logarithm to 6.02 log CFU/mL by the end of the analysis (Fig. 2). These results indicate that the *Lpb. plantarum* DA100 strain survived in vinegar throughout the 7-day period and managed to maintain the minimum number of viable microorganisms required for the product to be considered a probiotic during this time. This outcome could be attributed to the strain being isolated from pickles and the vinegar possibly responding quickly to stress factors such as low pH.

For the *L. fermentum* DA134 strain, the viability count decreased from 6.06 log CFU/mL to 5.02 log CFU/mL between the third and seventh days. The survival percentage of this strain significantly decreased from 84.51–70.01% by the end of the storage period (Fig. 2). Although the *L. fermentum* DA134 strain survived in vinegar by the end of the third day, it did not

survive until the seventh day. Consequently, it could not maintain the minimum viable microorganisms required for the produced vinegar to be called a probiotic during this time.

Many researchers have isolated LAB genera such as *Lactobacillus*, *Weissella*, and *Pediococcus* from traditional vinegar [2]. The *Weissella cibaria* DA28 strain, isolated from Tulum cheese and previously recognized for various probiotic properties [28], managed to maintain the desired viability until the end of the seventh day, with a fermentation completion of 6.00 log CFU/mL (Fig. 2). The survivability of this strain slightly decreased by the end of the seven days, concluding the analysis with 83.68% viability.

The *L. coryniformis* DA268 strain, isolated from bitter orange and previously identified to possess numerous functional properties, decreased approximately 2 logarithms by the end of the third day, resulting in a viability count of 5.16 log CFU/mL. This strain survived in vinegar until the end of the 7-day period; however, it did not maintain the minimum number of viable microorganisms required for the produced vinegar to be considered a probiotic.

[29] reported that using a mixed culture containing both *S. cerevisiae* and *L. plantarum* significantly improved the quality of citrus vinegar. According to [14], LAB increases the aroma components and affects the final product's technological properties and microbial stability by producing organic acids. In light of the current study, the selected LAB strains can effectively ferment rose vinegar as a suitable substrate and significantly enhance its quality. Additionally, it was observed that with an increase in storage time, a significant accumulation of metabolites reduced the components available for LAB in rose vinegar.

Acetic acid is the key component of vinegar fermentation, imparting its unique taste and aroma and being responsible for its fundamental sensory properties [30, 31]. In the present study, the AAB counts of the samples ranged from 4.76 to 6.54 log CFU/mL ($p < 0.05$). The highest AAB count of 6.54 log CFU/mL was observed in Group 1 at the end of fermentation. Group 2 and Group 4 exhibited significant changes in AAB viability between the initial and the seventh day (Fig. 3). The AAB count in Group 2 decreased from an initial count of 5.70 log CFU/mL to 3.54 log CFU/mL on the third day and further to 2.52 log CFU/mL on the seventh day. Similarly, Group 4 decreased from an initial count of 6.36 log CFU/mL to 3.62 log CFU/mL on the third day and further to 2.34 log CFU/mL on the seventh day. These results align with the findings of [30] and [2].

Yeasts metabolize carbohydrates into ethanol, carbon dioxide, and various secondary products, playing a significant role in alcohol fermentation. However, mold growth is undesirable during a healthy vinegar fermentation process. In the present study, the average yeast count for all groups was 3.30 log CFU/mL at the end of fermentation (Fig. 4). These findings are generally consistent with the yeast and mold count ranges reported by [32].

Physicochemical properties of rose vinegar

Table 2 presents the physicochemical properties of the rose vinegar groups. There were no significant differences between the initial and final pH values observed in all groups regarding the pH results. This finding suggests that pH may not be a reliable indicator for studying the short-term fermentation progression in rose vinegar samples. Furthermore, the pH results ranged from 3.59 to 3.68 at the end of fermentation. It is worth noting that although [30] reported higher pH values for traditional vinegar, the average pH of the rose vinegar in this study was determined to be above 3.60, which is higher than that of traditional vinegar.

Table 2
pH, total titration acidity, brix, color, total phenolic content and DPPH values of vinegars (initial, after 3 and 7 days of storage)

Analysis	Group name	24 h of storage	3 days of storage	7 days of storage
pH	Control	3.62 ± 0.01 ^{Ac}	3.62 ± 0.01 ^{Ac}	3.61 ± 0.00 ^{Ac}
	Group 1	3.62 ± 0.01 ^{Ac}	3.59 ± 0.00 ^{Bd}	3.59 ± 0.00 ^{Bd}
	Group 2	3.68 ± 0.01 ^{Aa}	3.68 ± 0.01 ^{Aa}	3.68 ± 0.01 ^{Aa}
	Group 3	3.65 ± 0.00 ^{Bb}	3.64 ± 0.01 ^{Bb}	3.67 ± 0.00 ^{Aab}
	Group 4	3.66 ± 0.01 ^{Ab}	3.62 ± 0.01 ^{Bc}	3.66 ± 0.01 ^{Ab}
Total titration acidity (%)	Control	2.50 ± 0.05 ^{Ba}	2.65 ± 0.05 ^{Ab}	2.69 ± 0.03 ^{Ab}
	Group 1	2.80 ± 0.09 ^{Aba}	2.63 ± 0.12 ^{Bb}	3.04 ± 0.24 ^{Aab}
	Group 2	2.76 ± 0.21 ^{Aba}	3.51 ± 0.22 ^{Bb}	3.27 ± 0.29 ^{Aab}
	Group 3	2.65 ± 0.52 ^{Ba}	358 ± 0.21 ^{Aa}	3.40 ± 0.34 ^{ABa}
	Group 4	2.99 ± 0.39 ^{Aa}	2.85 ± 0.13 ^{Ab}	3.40 ± 0.35 ^{Aa}
Brix (%)	Control	2.77 ± 0.04 ^{Bbc}	2.87 ± 0.04 ^{Abc}	2.87 ± 0.04 ^{Abc}
	Group 1	2.75 ± 0.04 ^{Ac}	2.77 ± 0.04 ^{Ac}	2.85 ± 0.04 ^{Ac}
	Group 2	2.92 ± 0.04 ^{Ba}	3.05 ± 0.05 ^{Aa}	3.05 ± 0.05 ^{Aa}
	Group 3	2.82 ± 0.04 ^{Babc}	2.92 ± 0.04 ^{Ab}	2.97 ± 0.04 ^{Aab}
	Group 4	2.87 ± 0.04 ^{ABab}	2.82 ± 0.04 ^{Bbc}	2.92 ± 0.04 ^{Abc}
L*	Control	25.21 ± 0.21 ^{Ab}	25.14 ± 0.27 ^{Ab}	25.18 ± 0.16 ^{Ab}
	Group 1	25.54 ± 0.01 ^{Aa}	25.44 ± 0.01 ^{Bab}	25.44 ± 0.00 ^{Ba}
	Group 2	25.36 ± 0.07 ^{Aab}	25.73 ± 0.28 ^{Aa}	25.39 ± 0.02 ^{Aa}
	Group 3	25.42 ± 0.03 ^{Bab}	25.39 ± 0.01 ^{Bab}	25.50 ± 0.02 ^{Aa}
	Group 4	25.55 ± 0.06 ^{Aa}	25.41 ± 0.06 ^{Bab}	25.39 ± 0.02 ^{Ba}
a*	Control	0.85 ± 0.06 ^{Aa}	0.80 ± 0.10 ^{Ab}	0.78 ± 0.01 ^{Aa}
	Group 1	0.83 ± 0.01 ^{Aa}	0.78 ± 0.02 ^{Bb}	0.72 ± 0.02 ^{Cab}
	Group 2	0.79 ± 0.01 ^{Ba}	1.01 ± 0.12 ^{Aa}	0.75 ± 0.05 ^{Bab}
	Group 3	0.83 ± 0.02 ^{Aa}	0.81 ± 0.02 ^{Ab}	0.68 ± 0.07 ^{Bb}
	Group 4	0.89 ± 0.11 ^{Aa}	0.75 ± 0.04 ^{ABb}	0.72 ± 0.01 ^{Bab}
b*	Control	2.46 ± 0.02 ^{Ac}	2.39 ± 0.00 ^{Ba}	2.38 ± 0.05 ^{Ba}
	Group 1	2.63 ± 0.01 ^{Aab}	2.51 ± 0.04 ^{Ba}	2.36 ± 0.02 ^{Ca}

Analysis	Group name	24 h of storage	3 days of storage	7 days of storage
	Group 2	2.52 ± 0.01 ^{Abc}	2.42 ± 0.40 ^{Aa}	2.37 ± 0.03 ^{Aa}
	Group 3	2.63 ± 0.01 ^{Aab}	2.52 ± 0.02 ^{Ba}	2.40 ± 0.02 ^{Ca}
	Group 4	2.75 ± 0.14 ^{Aa}	2.42 ± 0.01 ^{Ba}	2.40 ± 0.03 ^{Ba}
Total phenolic content mg GAE/L	Control	723.32 ± 0.01 ^{Be}	723.74 ± 0.00 ^{Ae}	720.33 ± 0.04 ^{Be}
	Group 1	950.20 ± 0.09 ^{Aa}	870.31 ± 0.10 ^{Bb}	791.96 ± 0.01 ^{Cb}
	Group 2	926.19 ± 0.03 ^{Ab}	891.19 ± 0.11 ^{Ba}	805.20 ± 0.06 ^{Ca}
	Group 3	857.49 ± 0.10 ^{Ac}	825.52 ± 0.23 ^{Bc}	788.17 ± 0.14 ^{Cc}
	Group 4	839.45 ± 0.14 ^{Ad}	815.41 ± 0.22 ^{Bd}	767.46 ± 0.20 ^{Cd}
DPPH μ m TE/ml	Control	13.384 ± 0.01 ^{Ae}	13.350 ± 0.00 ^{Bd}	13.307 ± 0.00 ^{Cd}
	Group 1	16.348 ± 0.01 ^{Aa}	14.919 ± 0.05 ^{Bb}	13.647 ± 0.01 ^{Cb}
	Group 2	16.026 ± 0.01 ^{Ab}	15.755 ± 0.01 ^{Ba}	13.832 ± 0.01 ^{Ca}
	Group 3	15.211 ± 0.01 ^{Ac}	14.327 ± 0.06 ^{Bc}	13.735 ± 0.03 ^{Cb}
	Group 4	14.755 ± 0.04 ^{Ad}	14.327 ± 0.12 ^{Bc}	13.405 ± 0.08 ^{Cc}

After the storage period, the titration acidity (TA) was measured between 3.04–3.40 g/L. The TA values of rose vinegar samples, produced using different LAB strains, varied significantly regarding vinegar variety and storage duration ($p \leq 0.05$). The control group, which did not utilize the LAB strain, exhibited the lowest TA level at the end of storage. Notably, in the present study, while no significant changes were observed in pH values during storage, a significant increase in titration acidity values was noted for both the control group and Group 3 vinegar samples ($p < 0.05$). Researchers have stated that this increase in TA value is primarily attributed to the production of acetic acid and other organic acids. In contrast, the pH stability may be due to the weak acidity of the organic acid [20].

The current study findings reveal that the brix values of the rose vinegar samples ranged from 2.85 to 3.05%. A significant difference was observed between the % brix values of the rose vinegar samples and the storage time ($p < 0.05$). According to previous reports by [1] and [30], the brix values of fruit vinegar ranged from 1.02–20.80%.

In the case of vinegar, color serves as a crucial indicator of quality [33]. The change in color of rose vinegar fermented with four LAB groups during the 7-day fermentation period is presented in Table 2. The rose vinegar samples with LAB additions exhibited L values ranging from 25.39 to 25.50, a value ranging from 0.68 to 0.75, and b values ranging from 2.36 to 2.40. The variations in color values were statistically significant for the vinegar group and the storage duration ($p < 0.05$). This may be attributed to the degradation of phenolic substances resulting from the pH drop during LAB fermentation of rose vinegar samples with a high red component. [34] reported that vinegar with a higher phenolic content typically displays lower whiteness/darkness values but higher red-component color values.

Total phenolic content of rose vinegar

The total phenolic content of vinegar was determined within the 723.32–950.20 mg GAE/L range. Initially, during fermentation, the phenolic content of the control group was lower than that of the vinegar samples with LAB-added groups ($p < 0.05$). [35] infused dried apples with probiotics and observed an increase in the amount of phenolic substances in their samples, which aligns with the findings of our study. A decrease in the amount of phenolic substances was observed in all

vinegar samples during the storage period. [30] produced grape, apple, artichoke, pomegranate, apple-lemon, and hawthorn vinegar using the traditional method. The total phenolic content of this vinegar was determined as follows: 75.01-2228.79, 40.44-434.88, 236.67, 257.53, 201.64, and 306.80 mg GAE/L, respectively. Two studies on rose vinegar reported total phenolic contents of 438.68 mg GAE/L and 855.64 mg GAE/L [36, 37]. The results of our study are consistent with the existing literature regarding the total phenolic substance content.

Antioxidant activity of rose vinegar

The DPPH values of the vinegar samples were determined within the range of 13.384–16.348 $\mu\text{m TE/mL}$. [37] reported rose vinegar's ORAC and TEAC values as 14.86 and 8.24 $\mu\text{m TE/mL}$, respectively. Upon the addition of probiotics, the phenolic content of the vinegar increased, leading to an observed enhancement in antioxidant activity. Generally, the total phenolic content of a product exhibits a direct relationship with its antioxidant activity [38]. This finding can be attributed to the effective binding of rose vinegar to cell tissues by the LAB strains used in the present study, consequently exerting a protective effect on phenolic compounds. The reduction in phenolic and antioxidant contents of rose vinegar samples after fermentation was associated with a decline in LAB count in rose vinegar samples during storage.

Organic acid compounds in rose vinegar samples

In the present study, eight different organic acids, namely acetic acid, propionic acid, succinic acid, oxalic acid, citric acid, fumaric acid, malic acid, and tartaric acid, were quantified initially and after seven days of storage (Fig. 5a and 5b). The primary organic acids detected in the vinegar samples were acetic, propionic, and oxalic. Acetic acid, the predominant organic acid in vinegar, imparts a mild spicy flavor to the palate [39]. In our study, the highest concentration of acetic acid was found in Group 4 at the end of the seventh day, with a concentration of 44.20 mg/100 mL, followed by Group 3 with 36.45 mg/100 mL. The production of organic acids can vary among different types of LAB (lactic acid bacteria) and even among individual strains within a species [40]. This variability was also observed in our study. Notably, Groups 1 and 4 exhibited relatively high levels of oxalic acid production, which can be attributed to the heterofermentative activities of LAB.

The yeast's production/consumption of organic acids during fermentation is mainly attributed to carbon metabolism. Furthermore, organic acids can be derived from the glyoxylate pathway and may serve as intermediates and/or by-products of glycolysis [41]. It is hypothesized that yeasts may have produced some organic acids detected in rose vinegar through these pathways. One such organic acid is citric acid. A study investigating citric acid production by yeast strains reported an average production of 25 g/L [42]. Although our study did not detect a significant amount of citric acid, it is plausible that yeasts contribute to its production in addition to LAB. [43] indicated that both yeasts and LAB could produce succinic acid, which aligns with our findings as we observed the production of this organic acid in all groups. Furthermore, a slight increase in the concentration of malic acid was observed from the initial 24 hours to the seventh day. This increase resulted in higher acidity in the vinegar, leading to a slightly sour taste.

Aroma profile of rose vinegar

A total of 16 different aroma compounds were identified in the vinegar samples (Table 3), with phenyl ethyl alcohol (PEA) determined as the major aroma compound. PEA is a colorless oily liquid with a mild and warm rose-honey-like odor, exhibiting moderate to weak strength. Its presence significantly influences the organoleptic properties of vinegar [44]. Furthermore, rose vinegar samples contained high levels of 1-Tetradecanol and 1-Hexadecanol, along with 1-Dodecene and n-dodecane, which are pleasant volatile compounds commonly associated with floral aromas and found in the composition of some essential oils [44, 45]. Over the storage period, rose vinegar supplemented with probiotics increased the levels of 1-Dodecene and n-Dodecene, aligning with the findings of [46]. Similarly, the levels of 1-hexadecanol, a volatile organic compound found in roses [47], and 1-Tetradecanol, associated with a fruity and coconut-like aroma [48], along with alcohol-derived compounds such as heptadecanol, hexadecanol, and n-tetradecane, increased at the end of the storage period. This observation suggests that probiotic strains contribute to the fermentation process during storage, resulting in higher concentrations of volatile alcohol compounds. Linalool, a functional monoterpene compound commonly found in citrus peel

oil and grapefruit oil, represents a prominent volatile component of essential oils and finds applications in healthcare products [49, 50]. In the present study, linalool in rose vinegar enhanced their bioavailability.

Table 3
Aroma volatiles found in vinegar samples (% of total area)

	24 h storage					7 days of storage				
	Control	G1	G2	G3	G4	Control	G1	G2	G3	G4
L-Linalool	1.66 ± 0.02	1.59 ± 0.20	1.68 ± 0.09	1.58 ± 0.11	1.59 ± 0.35	0.84 ± 0.12	0.99 ± 0.30	0.97 ± 0.18	1.05 ± 0.30	1.04 ± 0.29
Phenyl ethyl alcohol	62.37 ± 0.10	65.12 ± 0.18	61.65 ± 0.12	63.03 ± 0.23	70.91 ± 0.24	58.48 ± 0.35	59.29 ± 0.12	60.80 ± 0.19	57.89 ± 0.07	60.18 ± 0.14
Azulene	1.18 ± 0.03	1.13 ± 0.13	1.19 ± 0.17	1.12 ± 0.05	1.06 ± 0.10	0.79 ± 0.10	0.84 ± 0.14	0.83 ± 0.12	0.89 ± 0.12	0.84 ± 0.07
3-Octanol	0.97 ± 0.08	1.06 ± 0.02	1.05 ± 0.07	1.12 ± 0.21	1.26 ± 0.07	1.29 ± 0.16	1.48 ± 0.10	1.36 ± 0.19	1.26 ± 0.19	1.26 ± 0.18
1-Dodecene	10.26 ± 0.14	9.30 ± 0.07	11.21 ± 0.18	10.51 ± 0.04	10.27 ± 0.12	10.44 ± 0.14	10.87 ± 0.05	10.21 ± 0.11	12.11 ± 0.33	10.99 ± 0.12
n-Dodecene	1.66 ± 0.08	1.66 ± 0.19	1.82 ± 0.03	1.77 ± 0.08	1.86 ± 0.06	2.09 ± 0.22	1.88 ± 0.13	1.95 ± 0.18	2.26 ± 0.27	2.20 ± 0.30
α-Citronellol	1.52 ± 0.02	0.86 ± 0.27	0.56 ± 0.04	0.53 ± 0.19	0.86 ± 0.02	0.42 ± 0.09	0.74 ± 0.33	0.73 ± 0.10	0.58 ± 0.18	0.94 ± 0.12
Vetiverol	1.00 ± 0.14	0.40 ± 0.11	0.28 ± 0.19	0.33 ± 0.05	0.27 ± 0.08	0.25 ± 0.03	0.30 ± 0.38	0.29 ± 0.16	0.37 ± 0.19	0.26 ± 0.19
Buthoxyethoxyethyl acetate	1.00 ± 0.03	1.06 ± 0.16	1.12 ± 0.17	1.12 ± 0.16	1.19 ± 0.12	1.04 ± 0.24	1.19 ± 0.12	1.17 ± 0.29	1.26 ± 0.12	1.31 ± 0.22
1-Tetradecanol	8.73 ± 0.04	8.84 ± 0.15	9.81 ± 0.04	9.19 ± 0.02	0.93 ± 0.20	8.77 ± 0.20	9.88 ± 0.17	9.73 ± 0.06	10.53 ± 0.07	9.94 ± 0.10
n-Tetradecane	0.90 ± 0.17	0.86 ± 0.13	0.84 ± 0.11	0.79 ± 0.30	0.66 ± 0.06	2.51 ± 0.17	0.99 ± 0.08	0.92 ± 0.32	1.00 ± 0.16	0.84 ± 0.09
1-Hexadecanol	4.16 ± 0.14	4.12 ± 0.07	4.55 ± 0.08	4.46 ± 0.14	4.57 ± 0.05	5.01 ± 0.19	5.43 ± 0.04	5.84 ± 0.39	5.79 ± 0.22	5.23 ± 0.11
n-Hexadecane	0.49 ± 0.04	0.40 ± 0.16	0.49 ± 0.06	0.53 ± 0.07	0.53 ± 0.04	3.22 ± 0.05	0.79 ± 0.30	0.92 ± 0.08	0.95 ± 0.20	0.99 ± 0.15
1-Heptadecanol	1.87 ± 0.07	1.86 ± 0.05	2.03 ± 0.05	1.90 ± 0.15	1.92 ± 0.02	2.63 ± 0.12	2.96 ± 0.12	2.43 ± 0.02	2.42 ± 0.16	2.15 ± 0.17
n-Nonadecane	1.18 ± 0.04	0.66 ± 0.03	0.66 ± 0.09	0.72 ± 0.09	0.80 ± 0.15	0.96 ± 0.22	1.24 ± 0.38	0.68 ± 0.11	0.53 ± 0.19	0.68 ± 0.09
Other compounds	1.04 ± 0.12	1.06 ± 0.09	1.05 ± 0.22	1.31 ± 0.12	1.33 ± 0.11	1.25 ± 0.30	1.14 ± 0.21	1.17 ± 0.12	1.11 ± 0.10	1.15 ± 0.05

Sensory analysis results of rose vinegar

The sensory analysis scores of the rose vinegar samples are presented in Fig. 6 and Fig. 7. The aromatic intensity scores of the samples ranged from 5 to 8. Among the rose vinegars, Group 2 and Group 1 exhibited the most intense aroma ($p < 0.05$). Previous studies have shown that short-chain volatile organic acids can influence the acidity, aroma, and overall quality of vinegar [51]. Traditional vinegar production processes produce high ethyl acetate levels during alcohol fermentation [30, 52]. In this study, the evaluation scores for ethyl acetate odor ranged from 5 to 7, with Group 1 exhibiting the highest intensity.

This variation is attributed to the use of *Lpb. plantarum* culture in the production of Group 1. The sharpness scores of the vinegar samples ranged from 3 to 5. Group 3 and Group 4 vinegar samples had the highest total acidity values after fermentation and showed the highest sharpness. In contrast, the control sample with the lowest total acidity value displayed the lowest sharpness ($p < 0.05$). The sharpness values demonstrated consistency with the total acidity values. Vinegar derived from vegetable sources tends to exhibit lower sharpness than those produced from fruit. During vinegar fermentation, vinegar produced through efficient acetic acid fermentation may retain alcohol residues, imparting a wine-like character to the vinegar [27]. The evaluation scores for wine characters ranged from 2 to 4. Group 4 exhibited the highest wine character, while Group 1 and Group 3, which also had low phenyl ethyl alcohol production, displayed the lowest scores for this attribute at the end of fermentation ($p < 0.05$). These observed differences among the vinegar samples may be attributed to the varying efficiency levels of the strains used in production. When evaluating the yeast aroma and taste of the vinegar samples, generally low scores ranging from 2 to 3 were obtained ($p < 0.05$). This difference is believed to be due to the low yeast content in the roses used for production.

According to reports, vinegar is characterized by a predominant sour taste, followed by slight sweetness, saltiness, and bitterness, which arise from the interplay and balance of different flavor components [29]. The bitterness scores of the vinegar samples were low, ranging from 1 to 2. The control group exhibited the highest level of bitterness. The bitterness scores were similar to those reported in a study on commercial grape vinegar by [53]. The fluidity scores of the vinegar samples ranged from 6 to 7, with Group 2 and Group 3 demonstrating higher fluidity than the other groups. This difference may be attributed to exopolysaccharide (EPS) production variations by the strains used. Regarding overall acceptability, Group 1 was the most preferred vinegar ($p < 0.05$), followed by Group 3. Additionally, the high value of the b^* brightness parameter may influence the overall impression score of the rose vinegar produced in this study.

Conclusion

Probiotic LAB strains, namely *Lpb. plantarum*, *L. fermentum*, *W. cibaria*, and *L. coryniformis* were employed to produce rose vinegar. The fermented rose vinegar's physicochemical, microbiological, and sensory characteristics were evaluated during a 7-day storage period at 30°C. Including *Lpb. plantarum* in the rose, vinegar exhibited satisfactory viability counts for LAB and AAB. Overall, the fermentation process increased antioxidant activity and total phenolic compound content by metabolizing phenolics and producing lactic and acetic acid. Furthermore, analysis of organic acids and volatile compounds associated with aroma profiles in the rose vinegar samples indicated the presence of components with significant health effects. The utilization of LAB as an alternative approach to producing rose vinegar is noteworthy, as it contributes to the generation of bioactive products and enhances rose vinegar's functional and sensory attributes.

Abbreviations

AAB	Acetic acid bacteria
LAB	Lactic acid bacteria
GYC	Glucose-Yeast Extract-Calcium Carbonate
MRS	De Man Rogosa and Sharpe
DPPH	2,2-diphenyl-1-picrylhydrazyl
YETEM	Suleyman Demirel University Innovative Technologies Center Application and Research Center
TA	Titration acidity
PEA	Phenyl ethyl alcohol

Declarations

Credit authorship contribution statement

Pelin Ertürkmen: Data curation, Conceptualization, Methodology, Investigation, Formal analysis, Reviewing and Editing, Project administration. **Özcan Bulantekin:** Data curation, Conceptualization, Methodology, Investigation, Formal analysis, Supervision, Software, Writing- Original draft preparation, Reviewing and Editing, Project administration. **Duygu Alp:** Data curation, Methodology, Supervision, Software, Writing-Original draft preparation, Writing- Reviewing and Editing, Formal analysis.

Declaration of competing interest

We declare that we have no conflict of interest.

Data availability

The data that has been used is confidential.

Funding

This research received no specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Ethical approval

This article does not contain any studies with human or animal subjects.

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Figures

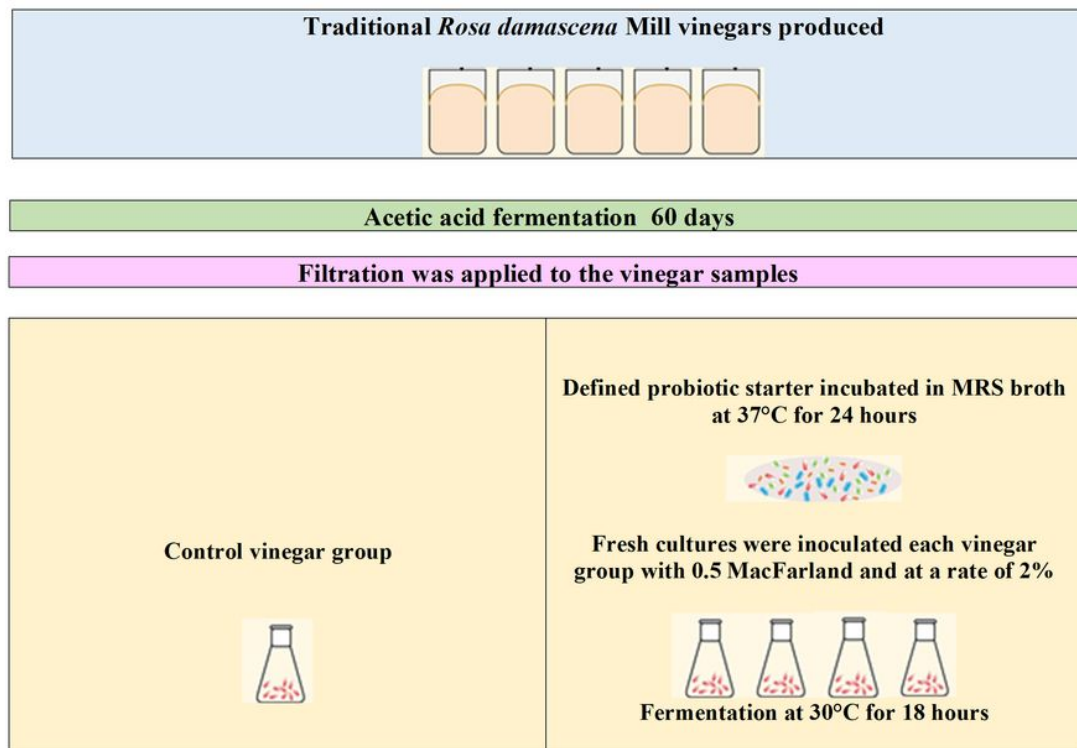


Figure 1

A schematic flow depicting the process for adding defined probiotic starter cultures with rose vinegar

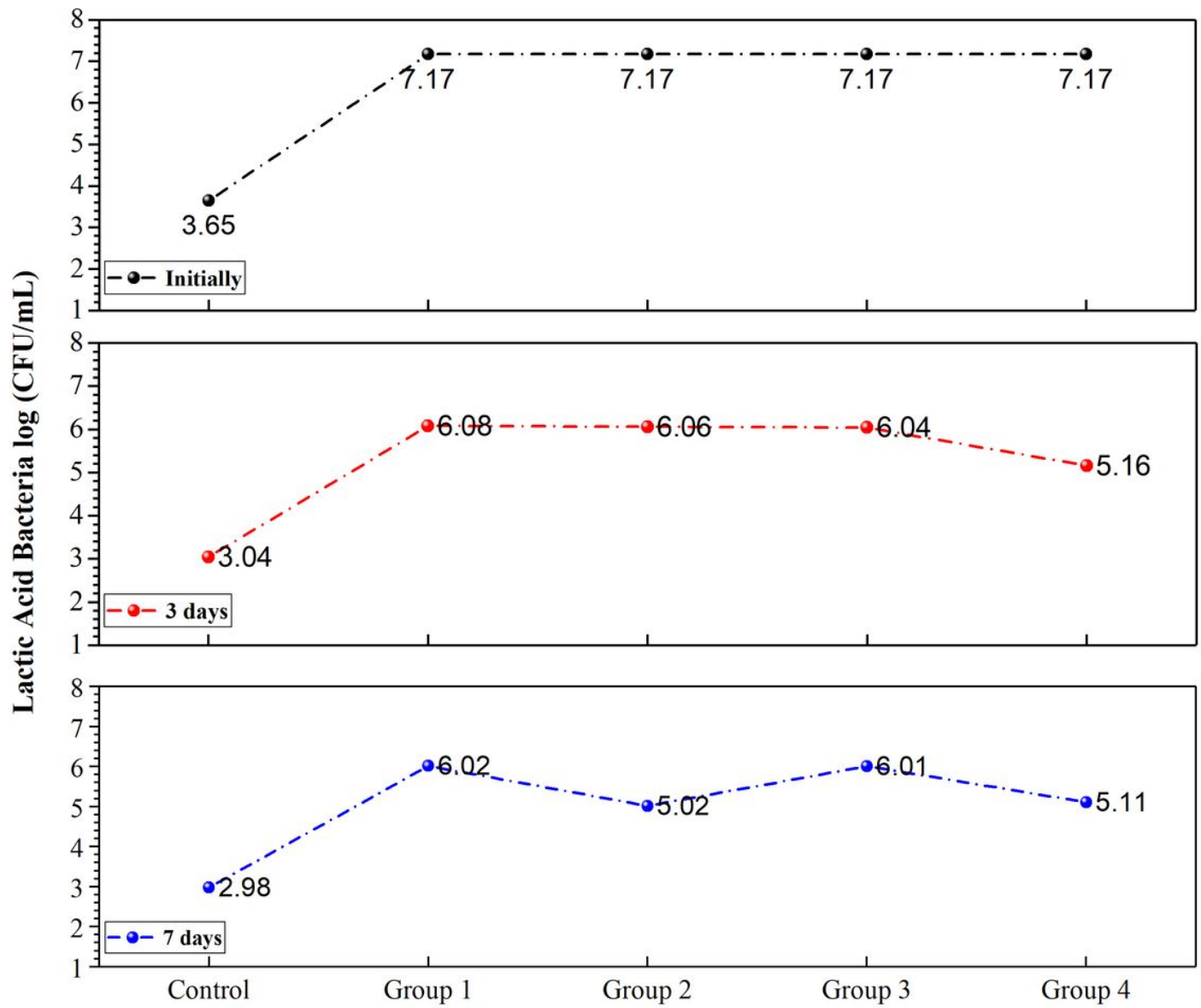


Figure 2

Viable cell count of LABs in all groups

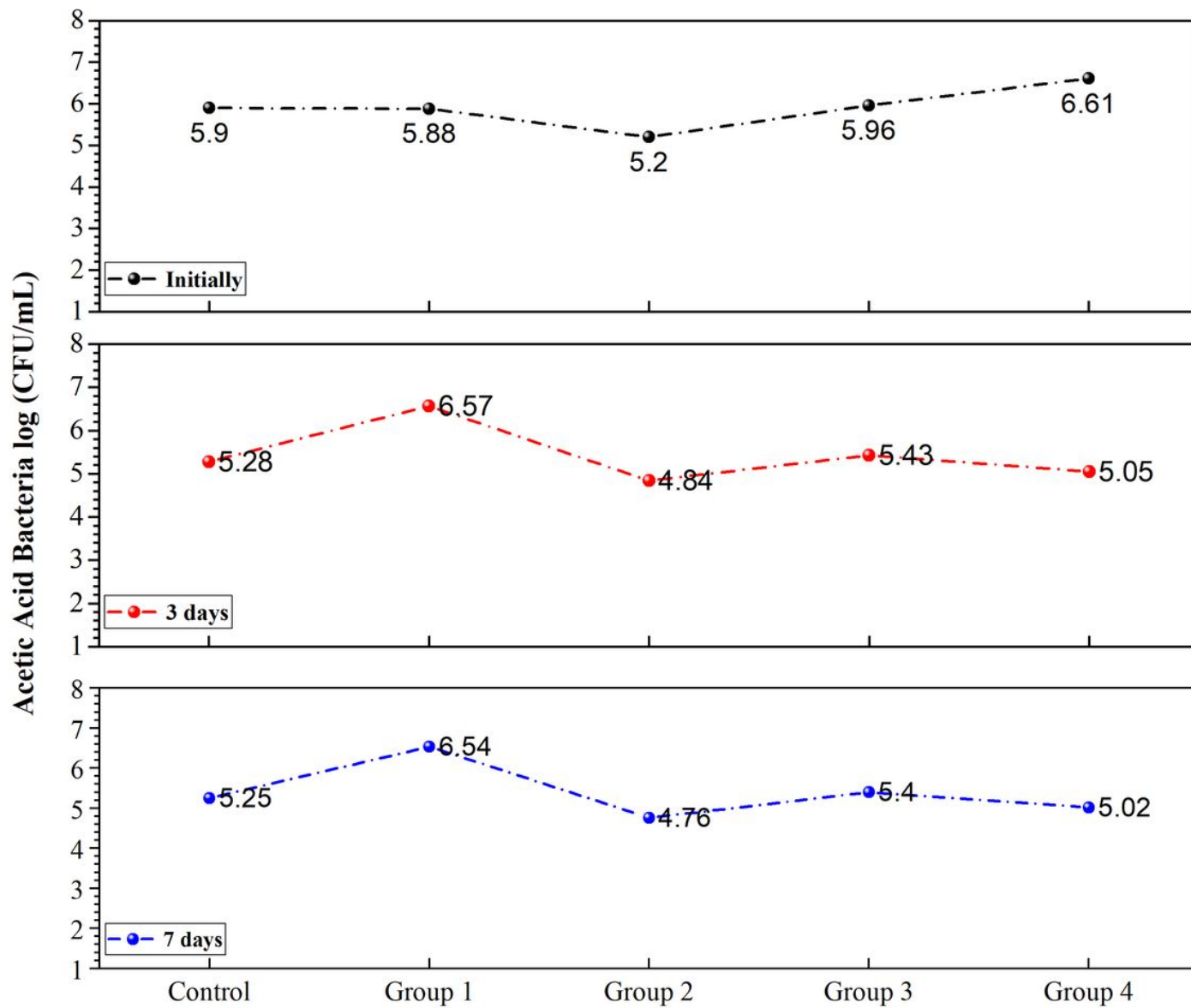


Figure 3

Viable cell count of AABs in all groups

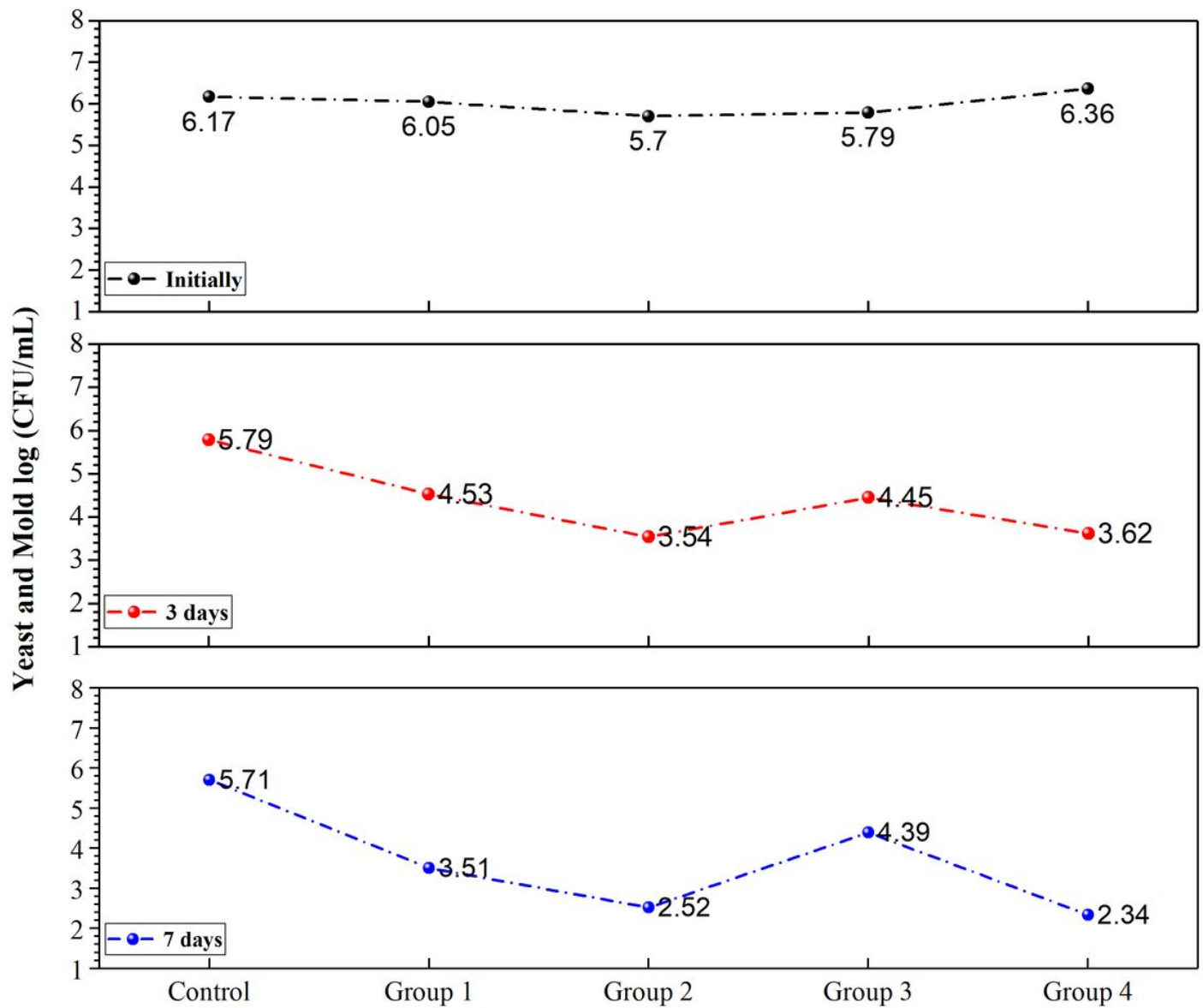


Figure 4

Viable cell count of yeast-mold in all groups

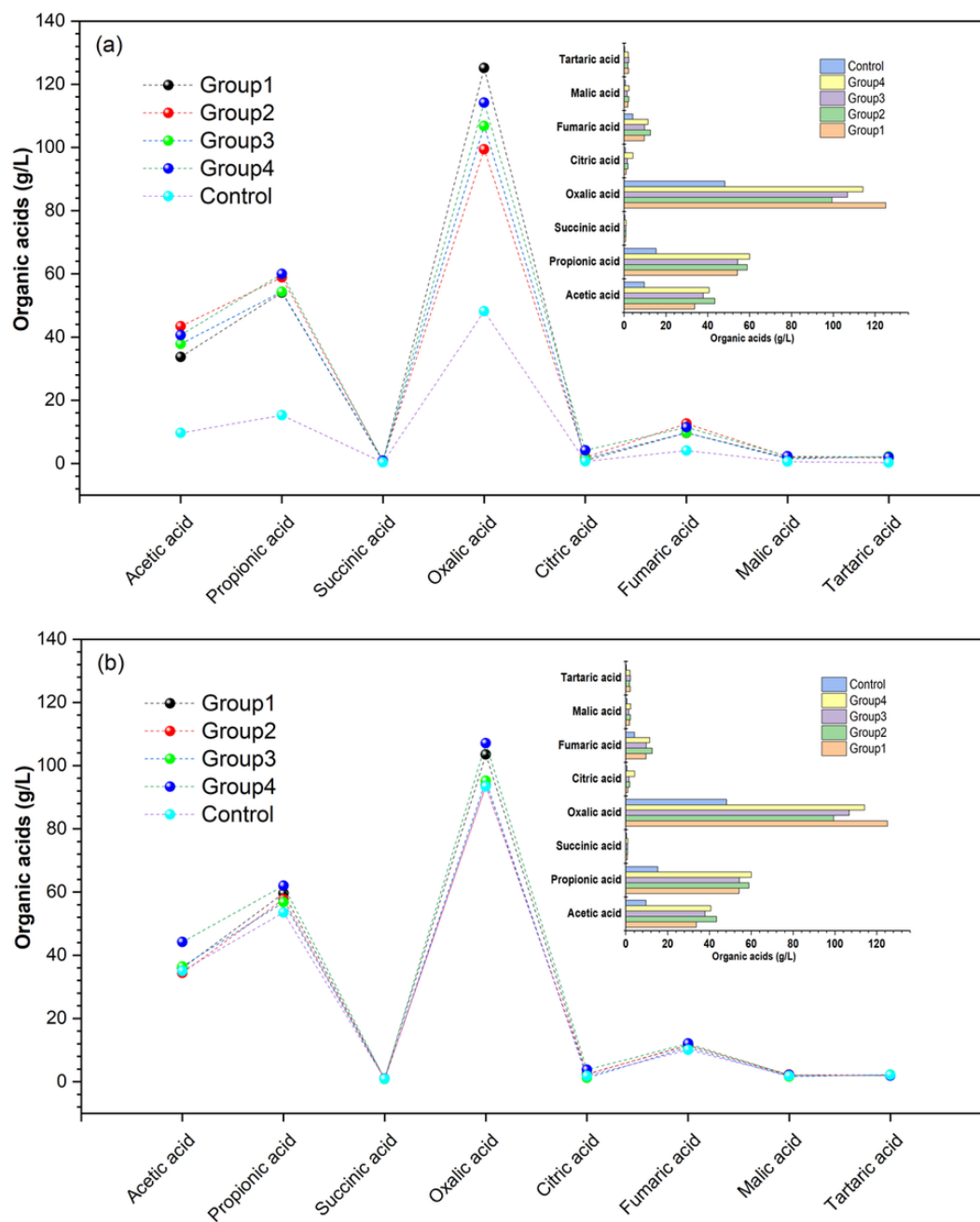


Figure 5

(a) Organic acids determined at the end of the 24th hour in all vinegar groups

(b) Organic acids determined at the end of the seventh day in all vinegar groups

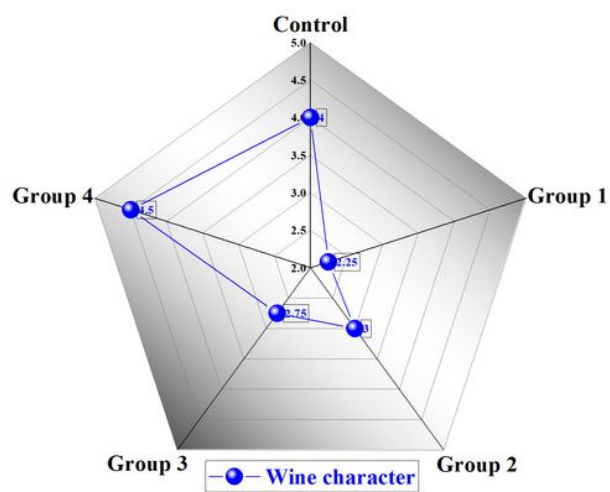
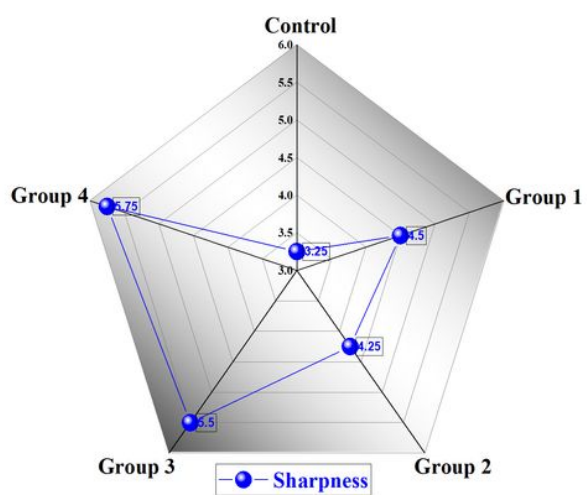
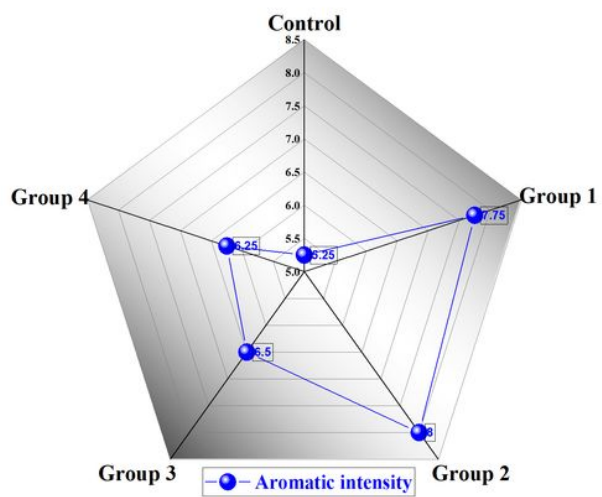
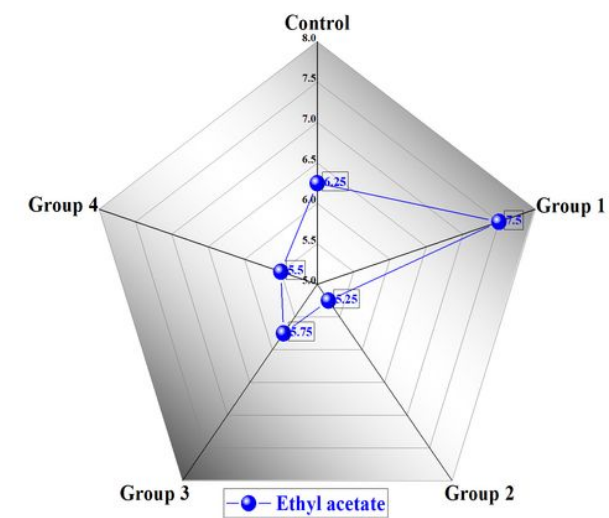


Figure 6

The sensory analysis scores of the rose vinegar samples

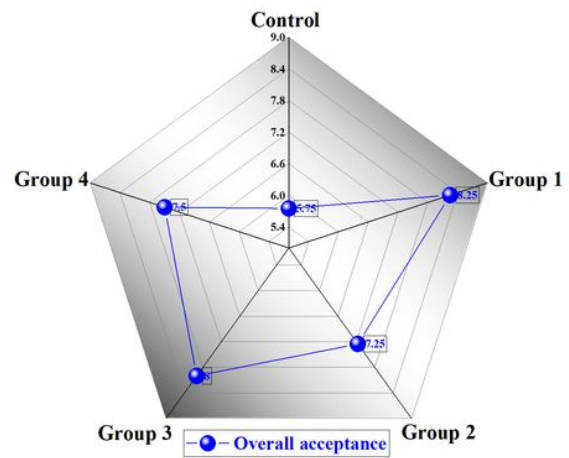
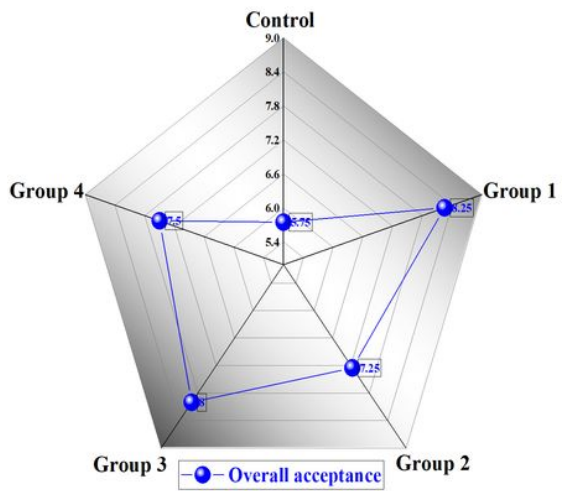
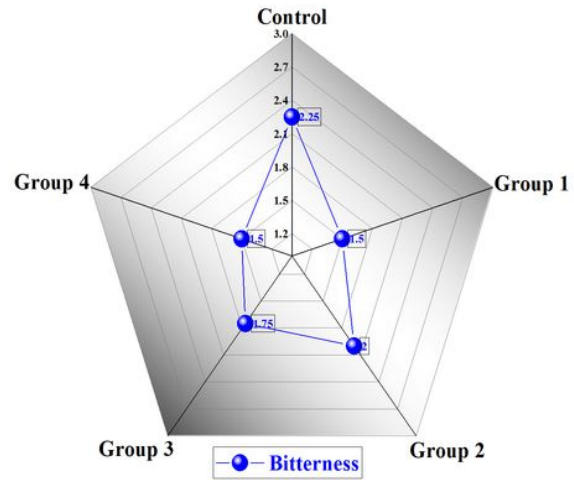
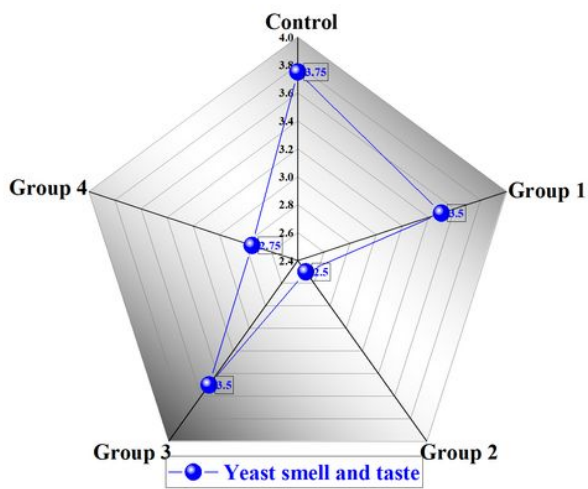


Figure 7

The sensory analysis scores of the rose vinegar samples